ISPH-0588

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No. : 09/918,026 Confirmation No.: 1035

Applicant : Crooke et al.

Filed : July 30, 2001

TC/A.U. : 1635

Examiner : T. Gibbs

Title : ANTISENSE MODULATION OF ACYL COA CHOLESTEROL ACYLTRANSFERASE-2 EXPRESSION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION PURSUANT TO RULE 1.132

Sir:

I, Mark J. Graham, residing at 2305 S. Ola Vista, San Clemente, California, 92672, a citizen of the United States of America, do hereby declare and state that:

1. I am one of the named joint inventors of the subject matter claimed in the above-captioned patent application. I earned a Bachelor of Science degree from Rockhurst University in 1982 and completed graduate courses in cytogenetics and recombinant DNA technologies at the University of Arizona in 1985. I have been employed at ISIS Pharmaceuticals since 1991 as a Scientist, Senior Research Associate, and presently as Assistant Director for Cardiovascular Antisense Drug Discovery.

2. This Declaration is submitted in the above-captioned application in response to the Examiner's rejections under 35 U.S.C. 102 (b) and (e) in the Office Action mailed November 18, 2004.

3. The Examiner relies upon four antisense oligonucleotide sequences, each presented in one of four (4) United States patents. These sequences are presented with varying oligonucleotide sugar chemistries, as follows:

U.S. Patent No.	Sequence	Corresponding ISIS Ref. Nos.
6,579,974	GGTCCACATCAGCACGTTCC	384603*
6,482,644	TGGTCATAGACCACCACGTC	103379*
6,180,353	CTGCAGAGGCCAGAAACACA	111221*
6,503,754	CACAGTCCATGGCCTGGGCA	119849*
6,579,974	GGTCCACATCAGCACGTTCC	384604**
6,482,644	TGGTCATAGACCACCACGTC	384598**
6,180,353	CTGCAGAGGCCAGAAACACA	384600**
6,503,754	CACAGTCCATGGCCTGGGCA	384605**
6,503,754	CACAGTCCATGGCCTGGGCA	384606***

* Corresponding ISIS oligonucleotides having 5-nucleotide 2'-O-methoxyethyl (2'MOE) wings, a 10-nucleotide 2'-deoxy gap, and phosphorothioate internucleotide linkages, *i.e.*, the chemistry used in the examples of the present application.

** Corresponding ISIS oligonucleotideshaving uniform 2'-deoxynucleotides and phosphorothioate internucleotide linkages.

*** Corresponding ISIS oligonucleotide having 5-nucleotide 2'O-Methyl wings, a 10-nucleotide 2'-deoxy gap, and phosphorothioate internucleotide linkages.

4. This Declaration presents data from experiments performed by me or under my supervision demonstrating that the antisense oligonucleotides relied upon by the Examiner to anticipate the pending claims fail to inhibit human acyl CoA cholesterol acyltransferase-2 (ACAT-2) expression by at least 60%.

5. In accordance with the procedures outlined in Examples 9 and 13 of the Specification, HepG2 cells were treated for twenty-four (24) hours with 150 nM of the antisense oligonucleotides identified in paragraph 3 above and evaluated by real-time quantitative PCT analysis for human ACAT-2 mRNA levels as compared to untreated control (UTC). The data

obtained for the four oligonucleotide sequences relied upon by the Examiner is expressed within the data table provided in Exhibit "A", attached hereto.

6. Data for the four oligonucleotide sequences relied upon by the Examiner presented in Exhibit "A" is also expressed graphically as human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) normalized percent control in Exhibit "B", attached hereto.

7. This data demonstrates that the antisense sequences relied upon by the Examiner in rejecting the claims under 35 U.S.C. 102 (b) and (e) fail to inhibit human ACAT-2 expression by at least 60%.

8. Of the oligonucleotide sequences relied upon by the Examiner, the 5-10-5 2'MOE gapmer of ISIS 384603 (Cases, et al.) produced the greatest inhibition (~52%). Each of the other oligonucleotide sequences relied upon by the Examiner yielded lower levels of inhibition. The 5-10-5 2'MOE gapmer of ISIS 384603 (Cases, et al.) is presented in Exhibit "A" in the row labeled for well "E3" and in Exhibit "B" to the far left.

In Exhibit "A", the four columns to the far right summarize the raw data found to the left. With reference well "E3", by way of example, the fourth column from the right (reflecting a value of ~49) provides the percentage of human ACAT-2 mRNA produced following oligonucleotide treatment. The third column from the right provides the percentage error calculated for the samples, *i.e.*, ~7. The second to last column provides the concentration of antisense oligonucleotide used, *i.e.*, 150 nM in the present example. The last column provides the ISIS reference number, *i.e.*, 384603 in the present example. This data is presented as the first bar in the graph shown in Exhibit "B".

Percent inhibition can be determined by subtracting the percent human ACAT-2 mRNA produced from 100%. In this example, inhibition is approximately equal to 100% - 48%, *i.e.*, 52% inhibition.

9. The last value presented in the table of Exhibit "A" as well as the last bar in the graph of Exhibit "B" is UTC (untreated control), and thus reflects 100% mRNA production or 0% inhibition.

Docket No. ISPH-0588 PATENT

11. I hereby declare that all statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 5/13/05 By: _____

Mark J. Graham